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HIV-1 reverse transcriptase and antiviral drug resistance. Part 2

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Structures of RT and its complexes combined with biochemical and clinical data help in illuminating the molecular mechanisms of different drug-resistance mutations. The NRTI drugs that are used in combinations have different primary mutation sites. RT mutations that confer resistance to one drug can be hypersensitive to another RT drug. Structure of an RT-DNA–nevirapine complex revealed how NNRTI binding forbids RT from forming a polymerase competent complex. Collective knowledge about various mechanisms of drug resistance by RT has broader implications for understanding and targeting drug resistance in general. In Part 1, we discussed the role of RT in developing HIV-1 drug resistance, structural and functional states of RT, and the nucleoside/nucleotide analog (NRTI) and non-nucleoside (NNRTI) drugs used in treating HIV-1 infections. In this part, we discuss structural understanding of various mechanisms by which RT confers antiviral drug resistance.

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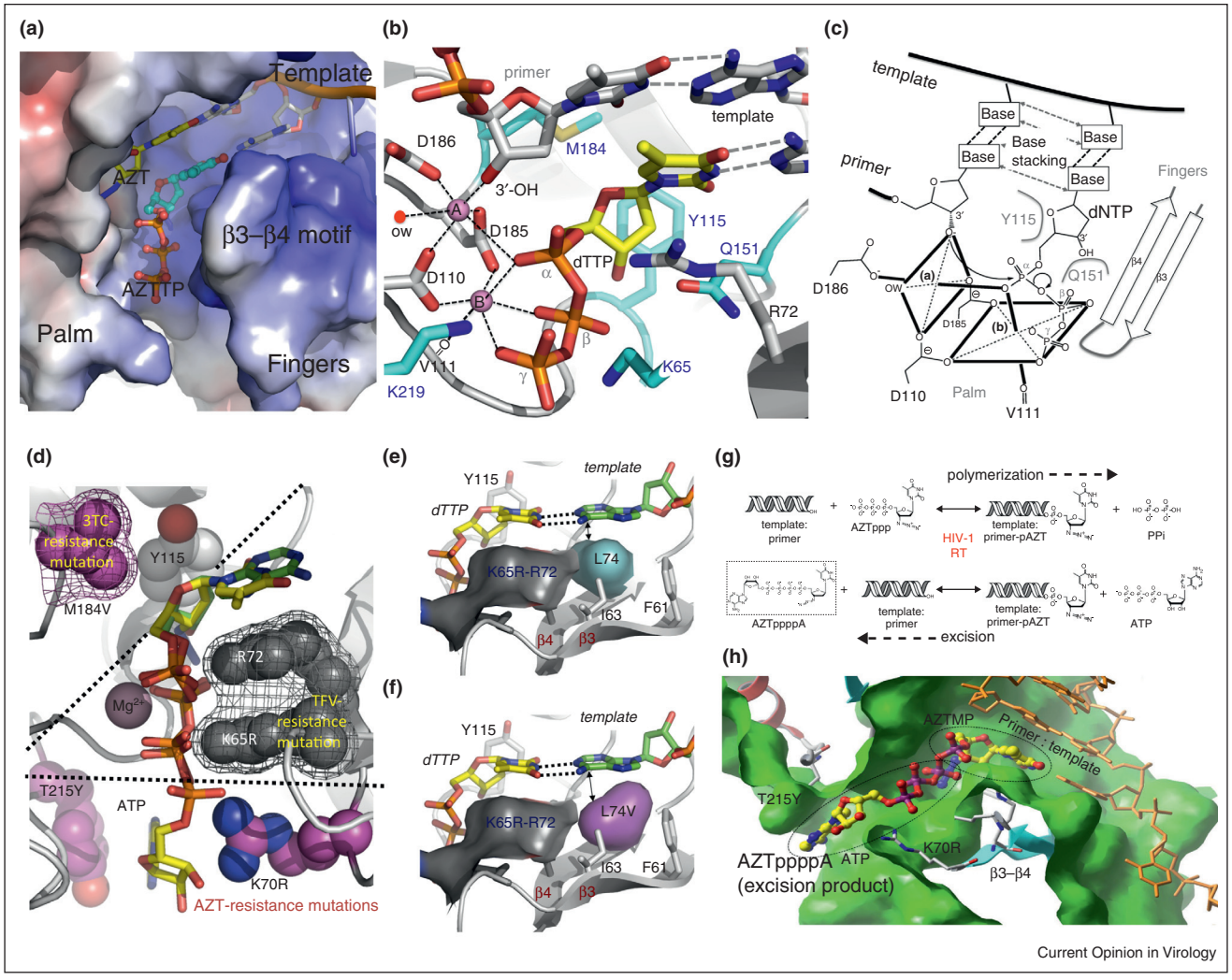
NRTI resistance by discrimination/exclusion

The dNTP substrates and analogs enter the open dNTP-binding cleft (Figure 1a), and the correct dNTP is selected for incorporation based on its complementarity (Watson–Crick base pairing) with the template overhang base (Figure 1b,c). The entire process starting from the entry of dNTP to the incorporation of a nucleotide is continuous and fast, although sorting of incorrectly paired bases limits the rate of incorporation. An NRTI-TP competes for RT binding with dNTPs; however, an NRTI-TP does not inhibit dNTP binding, and usually both bind RT at approximately micromolar affinity. HIV-1 inhibition by an NRTI occurs primarily when the NRTI is incorporated into the DNA primer. An NRTI-TP usually has modifications of its deoxyribose part when compared with a dNTP.

A dNTP (or NRTI-TP) undergoes association and dissociation of several molecular interactions with RT through the path starting from dNTP recognition by RT to nucleotide addition into a DNA primer strand. In the process, the distinct structural features of an NRTI-TP can be recognized by a specific mutation or sets of mutations that discriminate the drug from the analogous dNTP. For example, the active site mutation M184V/I causes high-level resistance to 3TC and FTC. A β-branched valine or isoleucine at the position 184 results in steric hindrance with the β-L-pseudo-ribose ring and thereby discriminates 3TC-TP (or FTC-TP) from dCTP [1,2]. Both 3TC and FTC inhibit hepatitis B, and the virus develops resistance to these drugs via analogous mutations in hepatitis B polymerase [3]. The HIV-1 RT mutation K65R emerges in response to treatments with TDF, ABC, ddI or d4T [4–6], and the mutation occurs at a higher frequency in subtype C HIV-1 [7]. The β3-β4 residue K65 interacts with the γ-phosphate of a dNTP in RT-DNA–dNTP ternary complex structures. Structures of K65R RT-DNA–dATP (or TFV-DP) [8•] revealed that the guanidinium side-chain functionality introduced by the K65R mutation stacks with the guanidinium group of R72, a key conserved residue that is involved in dNTP binding. The K65R-R72 guanidinium platform (Figure 1d) helps discriminate TFV-DP from dATP. Both wild-type and the mutant RT incorporated dATP and dATP-α-S at a similar rate indicating no elemental effect by K65R mutation, that is, the mutant RT discriminates at a conformational step rather than at the chemical step of NRTI incorporation [8•].

The positioning of the sugar moiety of a dNTP/NRTI-TP at the N site is primarily defined by its interactions with amino acid residues R72, Y115, and Q151. The conserved residue R72 helps dNTP binding by stacking with the base and interacting with the α-phosphate. The mutation Y115F emerged in response to ABC in mono- or combination therapies [9]. The mutation Y115F in combination with K65R decreases TDF susceptibility [10]. Residue Y115 is positioned underneath the sugar moiety and supports dNTP binding (Figure 1d). The Y115F mutation, which would retain its role in dNTP binding, acquires the ability of NRTI discrimination; wild-type hepatitis B polymerase has a phenylalanine at the equivalent position [3]. RT mutation at a nearby position, L74V, confers resistance to ddI [11,12]. Residue L74 is positioned under the base of the first template overhang and supports base-pair formation with an incoming dNTP (Figure 1e). Modeling of the L74V mutation [8•] suggests that the mutation apparently weakens the template:dNTP base-pairing and/or causes repositioning of

Figure 1



NRTI-TP (or dNTP) binding and mechanisms of NRTI resistance. **(a)** Positioning of an AZTTP molecule in an open dNTP-binding cleft of RT (represented by the electrostatic potential surface) in an RT-DNA complex [68••]. **(b)** RT-DNA–dTTP polymerase complex before catalysis was modeled using structural information from RT-DNA–dTTP complex [1], RT-ATP (a non-productive complex) [69], and RT-DNA–AZTTP complexes [68••]; the surrounding residues (cyan) can mutate to confer resistance to NRTIs. **(c)** A schematic representation of dNTP binding with the help of base-pairing, base-stacking, interaction with RT, and metal chelation; the catalytic reaction of DNA polymerization proceeds with an octahedral coordination environment for two Mg^{2+} ions. **(d)** An active-site superposition of K65R RT-DNA–dATP [8•] and EEM RT-DNA–AZTppppA complexes [23••] shows the relative locations of three distinct sites that confer resistance to three classes of NRTIs (3TC/FTC, TDF, and AZT). The 3TC-resistance mutation introduces a β-branch, TDF-resistance mutation K65R forms the K65R-R72 guanidinium platform, and primary EEMs T215Y and K70R help binding of an ATP molecule as the excision agent; mutations at one site, in general, are incompatible with mutations at the other two sites which provides a partial structural/biochemical basis for synergy between NRTIs. **(e)** Molecular surface representing the support to template–dNTP base pair provided by R72 and L74 side-chains. **(f)** An L74V mutation would disrupt the platform, and the mutation is incompatible with the K65R induced K65R + R72 part of the platform. **(g)** A schematic representation of catalytic reaction of polymerization versus excision by HIV-1 RT [23••]. **(h)** Binding of AZTppppA, the ATP-mediated excision product of AZTMP, to HIV-1 RT (shown as green molecular surface) in complex with DNA [23••].

dNTP by reducing the support to the template base (Figure 1f).

Residue Q151 interacts with the guanidinium group of R72 [8•]. The fact that the Q151 side chain in wild-type RT is unable to discriminate between a dNTP and a dideoxynucleoside-TP suggests that there may not be a stable interaction between Q151 and the 3′-OH of a dNTP. In contrast, Q151M mutation can cause resistance to dideoxynucleoside drugs. Q151M and four mutations (A62V, V75I, F77L, and F116Y), known as the 'Q151M complex,' co-emerged as a set in response to AZT combined with dideoxynucleoside drugs [13,14]. The Q151M complex causes resistance to almost all NRTIs except

3TC/FTC and TDF, and the viral strains carrying the mutations are termed multidrug-resistant (MDR) viruses. The specific structural basis of NRTI resistance caused by Q151M or Q151M complex RT has not been characterized; however, it has been shown biochemically that Q151M mutation discriminates NRTI-TPs [15–17], and the accompanying four mutations in the Q151M complex enhance the discrimination ability and viral fitness [13,18]. RTs carrying Q151M mutation develop resistance by discriminating NRTI-TPs from dNTPs before incorporation [19].

NRTI resistance by excision

A set of RT mutations (M41L, D67N, K70R, L210W, T215Y, and K219Q), known as thymidine analog mutations (TAMs) or AZT-resistance mutations (AZTr) [20], emerged in patients as distinctive mutations leading to AZT resistance [20]. Later, these mutations were biochemically shown to help excise AZT after the drug was incorporated into the DNA primer [21,22]. Excision is the primary mechanism by which RT develops resistance to AZT. Subsequently, several additional mutations were found to assist excision of AZT or other NRTIs, and this list of mutations can be grouped as Excision Enhancing Mutations (EEMs) [23••]. RT has the ability to excise either a normal nucleotide or an NRTI from the 3′-end of a DNA primer by pyrophosphorolysis, a reverse reaction of DNA polymerization (Figure 1g). Wild-type RT can excise the primer terminal nucleotide using a pyrophosphate or an ATP molecule as the excision agent; however, an RT carrying EEMs (TAMs) has a significantly enhanced excision ability using cellular ATP molecules as the excision agent [24,25]. The mutations facilitate ATP-dependent excision, generating AZTppppA (Figure 1g) as the reaction product. Crystal structures of RT-DNA–AZTppppA and related complexes illuminate the molecular mechanism of ATP-mediated excision of AZT (Figure 1g,h) [23••,26]. In agreement with an earlier hypothesis [25], the RT-DNA–AZTppppA structures revealed that the mutations help create an ATP-binding pocket adjacent to the dNTP-binding cleft. The T215Y mutated aromatic side chain stacks with the base while the K70R mutated side chain forms polar interactions with the α-phosphate and 3′-OH of ATP. The structural information revealed that the primary AZT-resistance mutations K70R and T215Y [27,28] play the most significant roles in ATP binding. The β-γ-diphosphate moiety of ATP mimics a pyrophosphate that chelates the two active site Mg^{2+} ions and triggers pyrophosphorolysis by RT to remove AZT-MP from the DNA primer terminus. The mutation-induced pocket has the potential to bind all NTPs and dNTPs; however, ATP is primarily used as the excision agent *in vivo* because: firstly, pyrophosphate is present at relatively low concentration in a cell; secondly, a dNTP would favor binding at the N site to form a polymerase catalytic complex rather than an excision complex; thirdly, an NTP with a purine base would have more extensive stacking with T215Y than one with a pyrimidine base; GTP was also shown to be an efficient excision substrate [22]; and fourthly, the cellular concentration of ATP is about a order magnitude higher than that of GTP [29]. The remaining EEMs play supportive roles in enhancing excision and improving viral fitness on top of the primary EEM backgrounds [23••,30,31]. The mutations D67N, and K219Q [32–34] arise on top of the K70R background, whereas M41L and L210W mutations are associated with T215Y mutation [35,36]. The T69 insertions in the β3–β4 fingers loop in combination with TAMs allow RT to excise a broader range of NRTIs [37–40]. Also, RT containing the Δ67 deletion enhances ATP-mediated excision of AZT on top of the TAM background [41,42]; however, the Δ67 RT does not excise a wider range of NRTIs.

In a fascinating parallel development, the hepatitis C (HCV) virus RNA-directed RNA polymerase (NS5B) has been shown to be capable of catalyzing NTP-mediated nucleotide excision [43,44••]. Analogous to the NTP-mediated nucleotide excision reaction by HIV-1 RT, that produces a dinucleoside tetraphosphate, the excision product by HCV NS5B (RNA polymerase) has ribonucleosides on both ends. Using a kinetic study, Jin et al. described that HCV polymerase excises chain-terminators and mismatched NMPs from 3′-end of a growing RNA primer [44••]. This result suggests that HCV and other polymerases may be using excision as a general fidelity mechanism. A lower rate of misincorporation by HIV-1 RT *in vivo* than free RT, as discussed in Part 1, may indicate that RT in infected cell conceivably may be using excision to enhance fidelity.

Cooperative/antagonistic mutations and NRTI combinations in therapy

In general, members of a class of inhibitors such as NNRTIs or PIs, that share a common binding site and mode of inhibition, do not show synergistic inhibition if combined. In contrast, many combinations of NRTIs have synergistic impact in inhibiting HIV-1 in patients. The NRTI synergies were found empirically from virological investigations and from clinical data; however, effective modeling and analysis of empirical data [45••,46•], and biochemical and structural studies help in understanding the complex synergistic relationships and their molecular basis. From a simplified structural and biochemical perspective, a synergistic relationship between two NRTI drugs can be influenced by the antagonistic relationship among the primary resistance mutations to the NRTIs. Primary NRTI-resistance exclusion mutations appear along the dNTP-binding track, or EEMs emerge at sites adjacent to the dNTP-binding track to facilitate excision. The sites and molecular mechanisms of resistance to one subclass of NRTIs are different than those to another subclass of NRTIs [8•];

the primary resistance mutation sites are distinct for 3TC, TDF, and AZT (Figure 1d). Resistance mutations to one subclass of NRTI can be positively or negatively associated with the resistance mutations to another subclass. The K65R + M184V double mutant is primarily detected in patients treated with a TDF, 3TC, and ABC combination [47]. The double mutant has increased resistance to 3TC and FTC, decreased rate of nucleotide incorporation, and increased susceptibility to TDF [17,48]. The β-branch introduced by the M184V mutation, and the K65R-induced R72-K65R guanidinium platform, form two walls on either side of the sugar moiety of a dNTP/NRTI-TP at the N site (Figure 1d). Thereby, the K65R + M184V mutations create structural constraints [8•] that firstly, still can accommodate the compact acyclic methoxypropyl moiety of TFV-DP, secondly, add restraint to dNTP binding that would slow down nucleotide incorporation, and thirdly, are highly discriminatory against the β-L-pseudo-ribose ring of 3TC (or FTC)-TP. Even though both K65R and M184V are exclusion mutations that co-emerge, a lower replication fitness and increased susceptibility to TDF may account for the clinical benefit of combining TDF and 3TC (or FTC).

Among other exclusion mutations, K65R is negatively associated with L74V [49,50] or most EEMs [51], and the combinations emerge less frequently. The mutations K65R and L74V are incompatible because the K65R-R72 platform and L74V-modified surface do not provide optimal structural support to dNTP-template base pairing [8•] (Figure 1e,f); K65R + L74V double mutant RT has a significantly reduced rate of nucleotide incorporation [49]. Also, L74V mutation decreases AZT excision [11,52]; probably by perturbing the positioning of AZT (at the DNA primer end) at the N site even when EEMs facilitate ATP binding. The multidrug-resistant Q151M complex in association with other exclusion mutations K65R or K70T/S/Q increases resistance to TDF while remaining resistant to dideoxynucleosides [53,54].

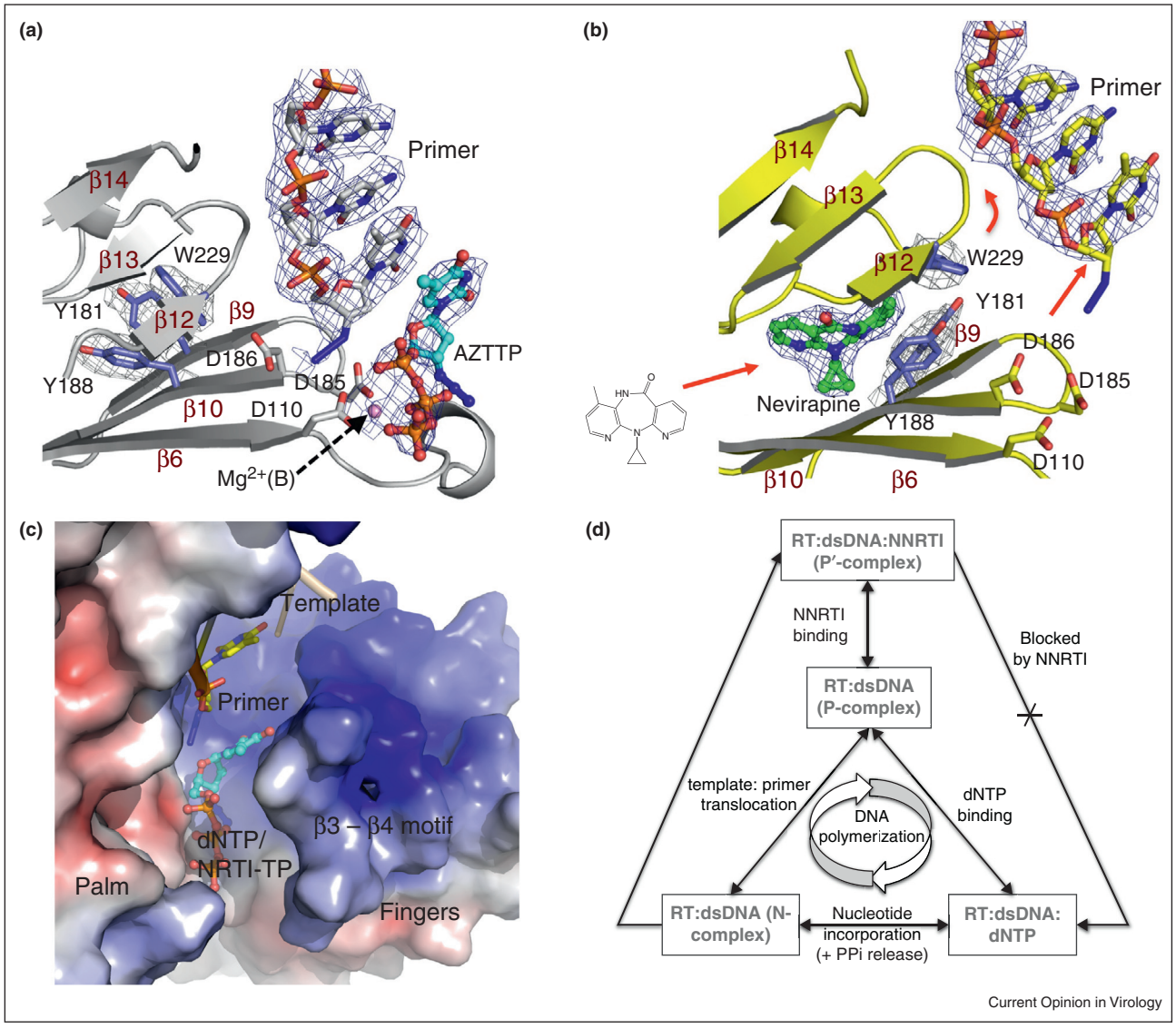
In general, exclusion mutations and EEMs are antagonistic [55–57]. Structurally, the K65R + R72 platform would interfere with proper positioning of the β-γ-pyrophosphate moiety of ATP, which is essential for excision [8•] (Figure 1d). Similarly, K70R and K65R would perturb the positioning of the triphosphate moiety of a dNTP and interfere with polymerization. Unlike the EEM K70R, all other mutations K70E/Q/S/T at position 70 function as exclusion mutations because these mutated side chains would not help ATP binding; however, the mutations may reposition or sense the γ-phosphate of an incoming NRTI-TP differently from that of a dNTP and invoke NRTI resistance by an exclusion mechanism. The mutation K70E is also antagonistic with EEMs [58], because the negatively charged shorter side chain of K70E is likely to interfere with the binding or positioning of ATP (Figure 1d,h). M184V/I mutation is antagonistic with excision. M184V/I mutation, which reduces the binding of 3TC (or FTC) at the N site, also may hinder the positioning of an NRTI-terminated 3′ end of a DNA primer at the N site for excision, that is, 3TC resistance enhances AZT susceptibility which favors the synergistic impact of combining AZT and 3TC [59].

RT inhibition by NNRTIs

RT inhibition by NNRTIs is indirect. Early structures of RT and RT-NNRTI complexes showed that NNRTI binding traps RT in a rigid conformational state with an open nucleic acid-binding cleft; however, the impact of NNRTI binding on the binding of nucleic acid or dNTP, and above all, the mechanism of NNRTI inhibition of DNA polymerization remained elusive. Pre-steady-state and steady-state kinetic experiments in the mid-1990s suggested that the binding of an NNRTI inhibits the chemical step of DNA polymerization while dsDNA and dNTP remain bound to RT [60,61]. A single-molecule FRET study revealed that RT frequently flips and slides over a double-stranded nucleic acid substrate [62••,63]; binding of dNTP stabilized the RT-DNA complex in a polymerase-competent mode while binding of an NNRTI induced destabilizing effects causing increased dissociation/association of RT with a double-stranded nucleic acid. The postulated mechanisms of inhibition by an NNRTI were: firstly, restriction of thumb mobility [64], secondly, distortion of the catalytic triad [65] that would block the chemical step of DNA polymerization by RT [60], thirdly, blockage of conformational state transition and not the chemical step leading to nucleotide incorporation [66], fourthly, repositioning of the primer grip [67], and fifthly, loosening of the thumb and fingers clamp [62••]. Multiple mechanistic hypotheses evolved because NNRTI binding causes multiple structural and conformational changes in RT, and the direct and indirect contributions of individual changes toward the NNRTI inhibition were not clear.

The hinge motion between the thumb and palm is essential for the translocation of RT along the nucleic acid following each nucleotide incorporation. The breathing space that is transiently created traps an NNRTI. Upon binding of an NNRTI, RT loses its conformational mobility to carry out nucleotide addition and translocation. The NNRTI pocket does not exist in any RT structure that does not contain a bound NNRTI. A direct consequence of NNRTI binding is the opening of the NNRTI pocket to accommodate the inhibitor. The pocket formation requires the β12–β13–β14 sheet, which contains the 'primer grip', to move away from the β6–β10–β9 sheet which contains the catalytic triad (D110, D185, and D186). The recent structure of RT-DNA–nevirapine complex provides a snapshot of the effects of an NNRTI on DNA polymerization [68••] (Figure 2a,b). The binding of nevirapine shifts the primer grip which

Figure 2



Structural basis for the inhibition of DNA polymerization by an NNRTI. **(a)** Structure of RT-DNA–AZTTP ternary complex obtained by soaking AZTTP into crystals of RT-DNA complex. **(b)** Soaking of nevirapine into the crystal created the NNRTI pocket, repositioning the 'primer grip' (on the β12–β13–β14 sheet) that moved the primer terminus away from the polymerase active site. **(c)** Electrostatic potential surface of RT bound to DNA and nevirapine. The crystallization experiments and structures showed an open dNTP-binding cleft into which dNTPs/NRTI-TPs can enter; however, structural perturbation by NNRTI binding did not allow a dNTP to chelate metals and form base-pairing and base-stacking interactions. **(d)** These structural constraints preclude the formation of an RT-DNA–dNTP polymerase competent (P) complex, rather forms a non-productive (P′) complex in the presence of an NNRTI — a structural basis for NNRTI inhibition [68••].

concomitantly displaces the primer 3′-end by ~5.5 Å away from its position at the polymerase active site. The interaction between the template-primer and the polymerase domain of RT is decreased upon nevirapine binding, which correlates with the earlier observation by a single-molecule study [62••]. The fingers subdomain has an open conformation that would allow the entry of dNTPs (Figure 2c); however, the repositioned template-primer would not permit the base-pairing or base-stacking to support the binding of a dNTP (Figure 1c) at the N site. This appears to be a primary reason why ordered binding of dNTP (or analog) to an RT-DNA complex was not observed in the presence of nevirapine whereas RT-DNA–dNTP (or analog) complexes could be formed when no NNRTI was present [68••]. These experiments were carried out in crystals that permitted rearrangements of the polymerase domain upon binding of a dNTP/analog or an NNRTI. Our attempts to form an RT-DNA–nevirapine–AZTTP complex yielded the structure of only RT-DNA–nevirapine complex, and

no well-defined binding of AZTTP at the polymerase active site when nevirapine is bound to RT [68••]. The structural information and published biochemical/biophysical results suggest that dNTPs may enter the dNTP-binding cleft (Figure 2c), interact with RT, and may induce conformational changes of RT like closing of the fingers; however, formation of a catalytically relevant RT-DNA–dNTP complex [1] would not be permitted when an NNRTI is bound. The repositioning of the template-primer by nevirapine binding disfavors the base-pairing or base-stacking of a dNTP at the N site. In addition to the base-pairing and base-stacking, metal chelation at the active site also contributes toward the binding of a dNTP substrate in a catalytically competent RT-DNA–dNTP complex (Figure 1b,c). None of the structures of RT-NNRTI binary complexes or the RT-DNA–nevirapine complex had any metal ion present at the polymerase active site, suggesting that a potential distortion of the catalytic site [65,69] by an NNRTI also forbids ordered binding of the triphosphate moiety of a dNTP because of the loss of Mg^{2+} ion chelation at the active site. Thereby, NNRTI binding prevents RT from achieving a conformational state of RT-DNA (or RT-DNA/RNA) complexed with dNTP that would be required for catalysis [66]. Additionally, the structure of RT-DNA–nevirapine complex showed that the thumb restriction [64] is induced by the primer grip repositioning, and the thumb and fingers clamp is loosened [62••] by the reduced interactions between the polymerase domain of RT and DNA. All of these effects of NNRTI binding force RT into a structurally and catalytically non-competent complex for dNTP binding at the N site and for polymerization (Figure 2d).

NNRTI resistance

Almost all of the NNRTI-binding pocket residues can mutate, and most of the mutations can confer resistance to NNRTIs. Some of the observed NNRTI-resistance mutations are L100I, K101E, V106A, K103N, V179D, Y181C, Y188L, G190A, and E138K (p51); K103N and Y181C are the most commonly observed mutations in patients exposed to NNRTI treatments. Residues K101, K103, and E138 (p51) are located at the rim of the pocket entrance for most NNRTIs. The remaining residues directly interact with an NNRTI in the pocket. The pocket mutations can cause firstly, loss of aromatic ring stacking interactions (Y181C or Y188L), secondly, steric hindrance (L100I or G190A/S), and thirdly, alteration of hydrophobic interactions (V106A or V179D). Impacts of NNRTI-pocket alteration are relatively severe on the rigid first-generation NNRTIs, for example, high level of resistance by Y181C to nevirapine. Substitution of G190 fills the central part of the pocket by which mutations at position 190 would reduce the binding of most NNRTIs; however, larger substitutions like G190E/Q severely impair the replication capacity of RT [70,71]; G190A is the most frequently observed mutation at position 190 because G190A mutation has the least impact on RT polymerase activity. A large side chain at position 190 would partly occupy the NNRTI-binding pocket and could conceivably function as a pseudo-NNRTI in reducing RT activity. A fitness cost is associated with certain pocket mutations or combinations of mutations if the virus has to develop resistance to more effective NNRTIs. Such NNRTIs will have to remain potent against all common NNRTI-resistance mutations that have minimum adverse impact on the viral replication.

Rim mutations at K101, K103, or E138 (p51) generally do not alter the bound states of NNRTIs. Two recent clinical studies showed that K103N and E138K mutations are predominantly associated with treatment failure of the efavirenz and rilpivirine, respectively, when combined with TDF and FTC [72•,73•]; M184V was the NRTI-resistance mutation that co-emerged in both arms of the study. The study indicated a comparable response by 25 mg/day of rilpivirine or 600 mg/day of efavirenz; rilpivirine was better tolerated, yet had a higher rate of virological failure. Efavirenz is the most widely used NNRTI, and K103N is the characteristic clinical resistance mutation that emerged upon treatments with efavirenz. In fact, the K103N mutation confers an almost uniform level of cross-resistance to most NNRTIs. In crystal structures of wild-type RT, the K103 side chain points outward and does not interact with a bound NNRTI. The K103N mutation apparently restricts access to the pocket for most NNRTIs. In absence of an NNRTI, the shrunken pocket accommodated a water molecule [74], whereas the K103N/Y181C mutant RT apo structure revealed that the solvent molecule was replaced by a sodium ion that had a distorted octahedral coordination involving the side chains of N103 and Y188, and the main-chain carbonyl oxygen of K101 and amide group of N103 [69]; the side chains of N103 and Y188 were also in position to make a hydrogen-bonding interaction. The difference in the energetic cost required for pocket formation by displacing the secluded water versus ion, or the extra hydrogen bond, may contribute to NNRTI resistance; however, the exact mechanism by which the K103N mutation develops resistance remains unclear. In rilpivirine, which remains effective against K103N mutation, the central pyrimidine ring interacts with the side chain of N103 [75]. A computational study using Monte Carlo/free energy perturbation calculations had suggested that NNRTIs that would form water-mediated hydrogen bonds at the entrance to the pocket, in particular with E138 (p51), would retain potency against K103N mutation [76].

The rim mutations K101E or E138K (p51) appear to have effects on NNRTI binding analogous to that of K103N. In wild-type RT, the side chains of E138 (p51) and K101 form a hydrogen bond that is lost in E138K or K101E mutant RTs [77]; the loss of the hydrogen bond was assumed to be the primary cause of NNRTI resistance

by E138K or K101E. If so, a mutant RT containing both E138K and K101E would restore the hydrogen bond between the two residues, and the double mutant RT would restore efficacy of NNRTIs. However, the double mutant showed a high level of NNRTI resistance and the E138D mutant RT that would not form the hydrogen bond with K101 remained NNRTI-susceptible just by retaining the charge of E/D138 side chain [78], which downweighs the suggested critical role of the hydrogen bond between K101 and E138 on NNRTI binding. A recent kinetic study indicated that rilpivirine had a higher rate of dissociation from E138K (p51) mutant versus wild-type RT [79•]. The cumulative biochemical and structural knowledge so far indicates that the rim mutations K103N and E138K primarily alter the association and dissociation of NNRTIs with RT. Even though E138K itself only causes a low level of resistance to rilpivirine, addition of M184V/I mutation enhances rilpivirine resistance, and E138K enhances the fitness of the virus on the M184V/I background, which reveals a synergistic relationship between the NNRTI- and NRTI-resistance mutations that benefits the virus [72•]. In contrary, the NNRTI-resistance mutation Y181C on a background of NRTI-resistance mutations decreases ATP binding as the excision substrate [80], indicating an antagonistic relationship.

Use of NRTIs and NNRTIs in combinations

Effective combinations of HIV-1 drugs are being optimized empirically based on clinical data. One or more NRTIs are used in almost all current clinical drug cocktails, and many combinations of NRTIs are synergistic. RT mutations that emerge along the dNTP-binding cleft to discriminate NRTI-TPs or to enhance excision of an NRTI are often incompatible for RT fitness, or mutations that develop resistance to one NRTI enhance sensitivity to another – among the molecular bases for the long-term clinical utility of NRTIs in combinations. Because of the different inhibition mechanisms, combinations of NNRTIs and NRTIs are effective in reducing viral replication. NNRTIs have the advantage of high specificity and consequently low side effects. An NNRTI is primarily used in combination with NRTI(s) in first-line therapies against HIV-1 infections; however, higher level of treatment failure has been observed for patients carrying traces of NNRTI-resistance mutations [81•]. NNRTIs are also important for blocking mother-to-child transmission, and NNRTIs are currently under clinical investigations as potential microbicides to prevent sexual transmission of HIV-1 [82]. The relationships among RT mutations are complex and are still evolving [46•]. New resistance patterns [83,84] emerge with the use of new drugs and combinations. No single experimental approach or analysis fully explains the complex phenomena associated with HIV-1 infection and drug resistance. Multiple experimental platforms, analysis of experimental data across the platforms, and their relationships with clinical observations are continually helping to unfold new details on the virus and antiviral resistance. Frequent evolution of new resistance mutation patterns and emerging side effects from long-term use of drugs underscore the constant need for new HIV-1 drugs.

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- • of special interest
- •• of outstanding interest

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